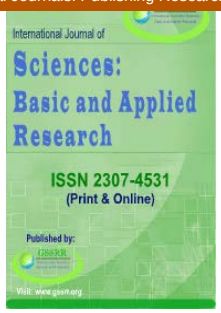
International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied



Evaluation of Extracts of Some Noxious Plants against Coffee Berry Disease (*Colletotrichum kahawae L.*)

Serawit Handiso[a*] , *Tesfaye Alemu*[b]

Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, P O Box, 1176, Addis Ababa, Ethiopia

[a]*Email: - serawithandiso@gmail.com*

[b] *Email: - tesfayealemu932@gmail.com*

Abstract

Aqueous, alcoholic and dry extracts of five different noxious plants, namely, *Senna occidentalis, Melia azendrachta, Parthenium hysterophorus, Calotropis procera* and *Argemone mexicana* were tested in *in vitro* condition with the purpose of evaluating their inhibiting effects against *C. kahawae.* Data were collected on radial growth and percent inhibition. ANOVA had indicated that the aqueous and aqueous extracts of *Melia azendrachta* gave highest zones of inhibition followed *by Senna occidentalis* with 76.02%, 59.95%, respectively. Alcoholic extracts *Calotropis procera* and *Senna occidentalis* gave the highest zones of inhibition with 72.75% and 63.62%, respectively. *Senna occidentalis* had superior inhibition effect in both aqueous and ethanol extracts when applied 24 hours after inoculation with 83.37% and 80.67%. However, the aqueous extracts of all the botanicals tested were superior to *Clotropis procera* when applied 12 hours before inoculation of the fungus. Except ethanol extract of *Senna occidentalis,* the other botanicals were not statistically different from the control. This indicated that there is relationship between the time of application and efficacy of the antifungal compounds from these botanicals. However, the aqueous extracts of the seeds of these plants gave a better inhibitory effect than the leaf parts. Dry extracts of *Senna occidentalis* followed by *Argemone mexicana* gave better inhibitory effect against *C. kahawae* with 76.37% and 70.55%, respectively. Moreover, the extracts of *Calotropis procera* and *Melia azendrachta* had been with a promisingly higher inhibitory effect in this study. Therefore, in vitro evaluation and testing of these botanical plant extracts have antifungal compounds which reduced the mycelia growth of *Colletotrichum kahawae.*

---

* Corresponding author Tel.: +251-09-1-373-3068, Fax:+251-111
Emailaddress: serawithandiso@gmail.com

Hence, further pot and field researches are deemed necessary on these botanicals for the effective control of *C. kahawae.*

Keywords*:* Aqueous extracts; *Colletotrichum kahawa;* Ethanol Extracts; inhibitory effect; noxious plants extracts.

1. introduction

Coffee is one of the most important trade commodities in the world next to petroleum [1]. Ethiopia is the home of arabica coffee [2] and the first and the fifth largest coffee producer in Africa and in the world, respectively [3]. Current contribution of coffee is more than 146.3 MT ($1.12bln) of the country's foreign exchange earnings [4], accounts for little under half of foreign exchange earnings and 54% of the GDP [4,5] and the rest is consumed locally. However, this economically important crop is prone to a number of diseases that attack fruits, leaves, stems and roots, and reduce the yield and marketability [6]. Among the many biotic constraints of coffee production in the country, coffee berry disease caused by Colletotrichum kahawae is the major one. The overall national average loss due to coffee berry disease is estimated to range 25-30%, which amounts to 600 million Ethiopian Birr, if not controlled by intensive fungicide spray [6]. Despite the countless attempts of coffee berry disease management through varietal resistance, cultural and pesticide control, information on the utilization of noxious plant extracts for control is not sufficient in our country. Hence, this paper is initiated with aim to evaluate the ◄ influence of some noxious plant extracts on the mycelia growth of Coffee Berry Disease (Colletotrichum kahawae) at in- vitro condition.

2. Material and Methods

2.1. Collection of Diseased Coffee Berries

Green infected berries with active black lesions were collected from susceptible plots in Dec, 2012 for use as the initial source of inocula. The berries were surface sterilized by immersing in 5% sodium hypochlorite solution for two minutes followed by rinsing with sterilized distilled water five times, each for about one minute, to eliminate surface contaminants. Using sterile forceps, each of the berries were picked up and placed on a moist tissue paper in a clean plastic box which was hermetically sealed and maintained for five days at room temperature, for initiation of sporulation on coffee berries.

2.2. Preparation of Culture Medium

Potato Dextrose Agar (PDA) was prepared by dissolution of commercially formulated dehydrated (powdered) PDA. The PDA powder was mixed with sterilized distilled water in a flask at the rate of 39gm/l and was heated until melting.

The medium was autoclaved at 121 ^{o}c for 15 minutes to sterilize the media. The sterilized medium was transferred to an isolation room, maintained under aseptic condition and allowed it to cool to about 40 ^{o}c.

Streptomycin sulphate powder was added to the molten nutrient media at the rate of 1gm/l to avoid bacterial contamination and the medium was poured into sterilized Petri dishes at the rate of about 20 ml/Petri dish and the medium was allowed to solidify.

2.3. Purification of Fungal Culture

The sporulated fungal cultures on the damped berries were sampled and examined under a stereomicroscope to check whether they are the CBD fungus or not.

- The sporulation of fungal culture was examined and evaluated under stereomicroscope. Then, the fruiting bodies (mycelia colonies) from the berries were picked out with a sterile needle and were placed on the prepared nutrient media in the Petri dishes and incubated in the inverted positions for 11 days in a cooled incubator adjusted to 23 ^{o}C. The plates were placed in the inverted position to prevent water condensation on the agar surface that could cause rotting of the plated fungus. After fungal colonies grew out into the agar and covered about two thirds of a Petri dish, they were further sub cultured on fresh PDA medium to obtain pure of the test pathogen for maintenance.
- Blocks of fungal colonies were cut out with a sterile surgical blade from the leading edge of the actively growing portion and were transferred to fresh agar medium and incubated in an incubator at 21^{O}C.

2.4. In Vitro Evaluation of Antifungal Activities of Plant Extracts

The effect of each plant extract was carried out according to [7] and [8]. About one ml of each extract was spread on the pre-solidified PDA contained in a Petri dish. The treatment without extract was served as a control. Then, five-millimeter fungal agar block was cut with sterilized blade from the center of active growth region (7 days old) and was placed at the center of a 9 cm diameter Petri dish.

The Petri dishes were kept at 21^{o}C. The radial growth of fungus for each treatment was measured at the right angles from each colony every 48 hrs after 5 days of inoculation for a week. The experiment was carried out in CRD replicated thrice. Percentage of radial growth inhibition by each plant extract was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Growth of Fungus in Control (mm) - Growth of Fungus in Extract (mm) X 100}}{\text{Growth of Fungus in Control (mm)}}$$

2.5. Preparation and Extraction of Plant Materials

The plants were collected from elsewhere around Wolaita area. The plants used were *Melia azendrachta*, *Senna occidentalis*, *Calotropis procera*, *Parthenium hysterophorus*, and *Argemone mexicana*. The plants were extracted through maceration as recommended by [8]. The aqueous extracts was prepared in 100/500ml (w/v) of

sterilized distilled water and shaken in orbital shaker in 130 rpm for 1 hour. The mixture was allowed to stand for 48 hrs and filtered using cheese cloth followed by filter paper (Whitman No. 1).

3. Results

3.1. Effect of Extraction Methods and Plant Species on Mycelial Growth Inhibition of Colletorichum kahawae

In vitro evaluation of antifungal activity of aqueous extracts of five noxious plants at 10% (w/v) against *Colletotrichum kahawae* was studied through measurements of radial growth of this pathogen. There was significant ($p<0.005$) interaction effect between type of medicinal plant used and method of extraction in inhibiting radial growth of C. *kahawae*. As a result, the effect of medicinal plants on radial growth of the test pathogen was presented for each method of extraction. Generally, extracts of all the tested medicinal plants except aqueous extracts of *Melia azendrachta* uninhibited the mycelia growth of C. *kahawae* compared to the untreated control (Table 1). The inhibitory effect of aqueous extracts of the five noxious plants ranged from 40% to 76.02%. The highest inhibition of was recorded on Petri dishes treated with *Melia azendrachta* (76.02%) followed by *Senna occidentalis* (59.95%).

Table 1. Effect of extraction methods and plant species on mycelial growth inhibition of *C. kahawae* after five days of incubation at 30^{o}C.

Method of Extraction	**Noxious Plant**	**Radial Growth(cm)***	**Inhibition (%)**
Aqueous Extract	*Senna occidentalis*	3.34a	59.95
	M. azendrachta	2b	76.02
	Calotropis procera	3.67a	55.99
	Parthenium hysterophorus	5a	40.05
	Argemone mexicana	5a	40.05
	Control	8.34a	-
		LSD = 3.66	
Ethanol Extracts	*Senna occidentalis*	2.67b	63.62
	M. azendrachta	4ab	45.5
	Calotropis procera	2b	72.75
	Parthenium hysterophorus	4.34a	40.87
	Argemone mexicana	4.34 a	40.87
	Control	7.34 a	-
		LSD = 5.22	

*Means with the same letter are not significantly different (α =0.05)

In the case of Petri dishes treated with ethanol extracted botanicals, inhibition percentages on the mycelia growth of *C. kahawae* ranged from 40.87% to 73.62%. The highest inhibitory effect was shown by *Calotropis procera* (72.75%) followed by *S. occidentalis*(63.62%). *Melia azendrachta* has shown the inferior result with 45.5%, although the least inhibitory effect was recorded on Petri dishes treated with *Parthenium hysterophorus* and *Argemone Mexicana* with 40.87% in each. In general, S. *occidentalis* had the highest inhibitory effect on aqueous extracts and the second highest inhibitory effect ethanol extracts on *C. kahawae*. However, *Calotropis procera* had shown significant inhibitory effect on C. *kahawae* only with ethanol extract.

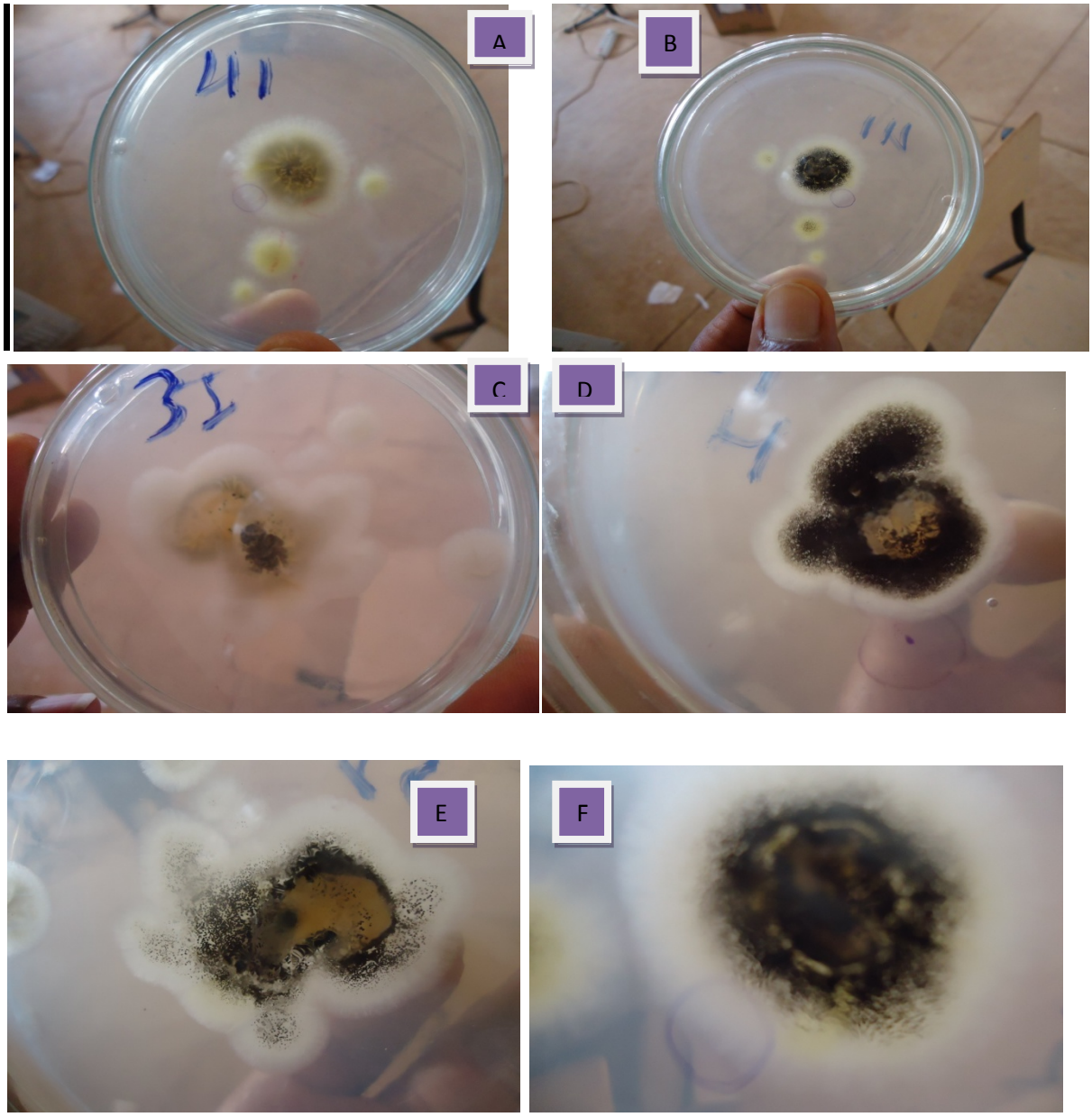


Figure 1. Petri dishes treated with extract of: A) *Senna occidentalis*, B) *Melia azendrachta*, C) *Calotropis procera*, D) *Argemone Mexicana*, E) *Parthenium hyestrophorus*, F) Control (untreated)

3.2. Evaluation of Extraction Methods, Plant Species and Time of Inoculation on Mycelial Growth Inhibition of C. kahawae

The influence of extraction methods, type of plant species used and time of inoculation on mycelial growth of *C. kahawae* was determined. Accordingly, the aqueous and ethanol extracts of the five botanicals were applied after 12 and 24 hours of inoculation. And the result showed that the aqueous extracts of all the botanicals applied 24 hours before the time of the treatments had depicted superior result over the control. *Senna occidentalis* was identified to have a higher inhibitory effect when applied 12 hours with 80.44%, as compared to 83.37 % in 24 hours of inoculation. Ethanol extracts of the plants evaluated at 12 hours before the placement of the fungus has shown that all of them were superior to the control (Table 2).

Table 2. Influence of extraction methods, plant species and time of inoculation on mycelial growth inhibition of *C. kahawae*

Method of Extraction	Plant Species	Time of Inoculation			
		12 hrs earlier		24 hrs later	
		Radial	Inh.**(%)	Radial	Inh.(%)
Aqueous extract	*Senna occidentalis*	1.5b	80.44	2b	83.37
	M. azendrachta	2.17b	71.71	2.67b	69.2
	Calotropis procera	3.67ab	52.15	3.67b	57.67
	Parthenium hysterophorus	1.67b	78.22	2.67b	69.2
	Argemone mexicana	2.34ab	69.49	2.34b	73.01
	Control	7.67a	-	8.67a	-
		CD = 5.55		CD = 4.91	
Ethanol Extracts	*Senna occidentalis*	1.34b	77.67	1.16b	80.67
	M. azendrachta	1.83b	69.5	2.83a	52.17
	Calotropis procera	1.67b	72.17	2.67a	55.5
	Parthenium hysterophorus	1.67b	72.17	2.16a	64
	Argemone mexicana	2.00b	66.67	2.16a	64
	Control	6.00a	-	6.00a	-
		CD = 3.86		CD = 4.33	

*Means with the same letter are not significantly different (α =0.05)

** Inh. = Inhibition

Furthermore, ethanol extracts of *Senna occidentalis* had performed best at 12 and 24 hours of the actual fungal placement with 77.67% and 80.67% inhibition, respectively. The overall result of the experiment has indicated that the application of the aquatic extracts these botanicals 24 hours after fungal inoculation gave the best result. On the other hand, the reverse is true alcoholic extracts of the five. This implies that both preventive and

curative treatments are more effective in reducing the mycelia growth of *C. kahawae* although the latter seemed to be more effective. This may be due to the highest rate of biodegradability of botanicals and earlier application would be more effective as compared to therapeutic treatments.

3.3. Effect of Extraction Methods and Parts of the Plant on Inhibition of Mycelial Growth of C. kahawae

The effect of extraction method and the parts of the plant showed that both the leaf and seed extracts of all the botanicals tested had shown superior inhibitory effect as compared to the control. Moreover, the seed parts in both aqueous and ethanol extracts had shown the highest inhibitory effect despite the fact that there was more statistical difference among the botanicals extracted from the seeds than the leaves.

Table 3. Effect of extraction methods and parts of the plant used on inhibition of *C. kahawae*

Method of Extraction	Plant Species	Plant Part Used			
		Leaf		Seed	
		Radial	Inh. (%)	Radial	Inh.(%)
Aqueous extract	*Senna occidentalis*	2b	75	1.34b	68.21
	M. azendrachta	2.5b	68.75	1.84b	74.98
	Calotropis procera	2b	75	1.5b	79.53
	P. hysterophorus	3.34b	58	2b	72.71
	Argemone mexicana	2.5b	68.75	1.67b	77.21
	Control	8.00a	-	7.33a	-
		CD =3.87		CD = 4.4	
Ethanol Extracts	*Senna occidentalis*	1.5b	52.68	1a	85.00
	M. azendrachta	1.67b	73.66	1.67a	74.96
	Calotropis procera	2b	68.45	2a	70.00
	P. hysterophorus	2.34a	63.1	1.67a	74.96
	Argemone mexicana	2b	68.45	1.67a	74.96
	Control	6.34a	-	6.67a	-
		CD = 4.09		CD = 5.72	

*Means with the same letter are not significantly different (α =0.05)

This shows that the inhibitory effects of the botanicals extracted from the seeds best exhibited in alcoholic extracts than aqueous extracts. The overall result also indicated that aqueous seeds *Senna occidentalis* extracted by using water (with 75%) and ethanol extracts of *Senna occidentalis* leaves, though superior to other botanicals, has shown inferior inhibitory effects in alcoholic seed extracts (which is 85%) and the inhibitory effect of the seeds of these botanicals was highest. However, aqueous, leaf extracts of all the botanicals under study had shown the least inhibition. The seeds and leaves of *Calotropis procera* in aqueous extracts had shown

the highest inhibition effects with 79.53% and 75%, respectively (Table 3). The results in this research showed that extracts of *S. occidentalis* gave significantly higher inhibition zone compared to all the tested plants.

3.4. Effect of Powdered Plant Extracts on Mycelial Growth Inhibition of C. kahawae

On another trial, the plant extracts were applied to see the effect of formulation on inhibition of *C. kahawae*. *Senna occidentalis* followed by *Argemone mexicana* had shown the highest inhibitory effect against *C. kahawae* with 76.37% and 70.55%. Furthermore, the activity of *Calotropis procera* was worth consideration though all the botanicals had shown superior results (Table 4).

Table 4. Effect of dry formulations and plant species on severity of coffee berry disease

Species	**Radial growth(cm)**	**Inhibition (%)**
Senna occidentalis	1.34a	76.37
M. azendrachta	2.34ab	58.73
Calotropis procera	2ab	64.73
Parthenium hysterophorus	2.34 ab	58.73
Argemone mexicana	1.67ab	70.55
Control	5.67bc	-
	LSD = 4.24	

*Means with the same letter are not significantly different (α=0.05)

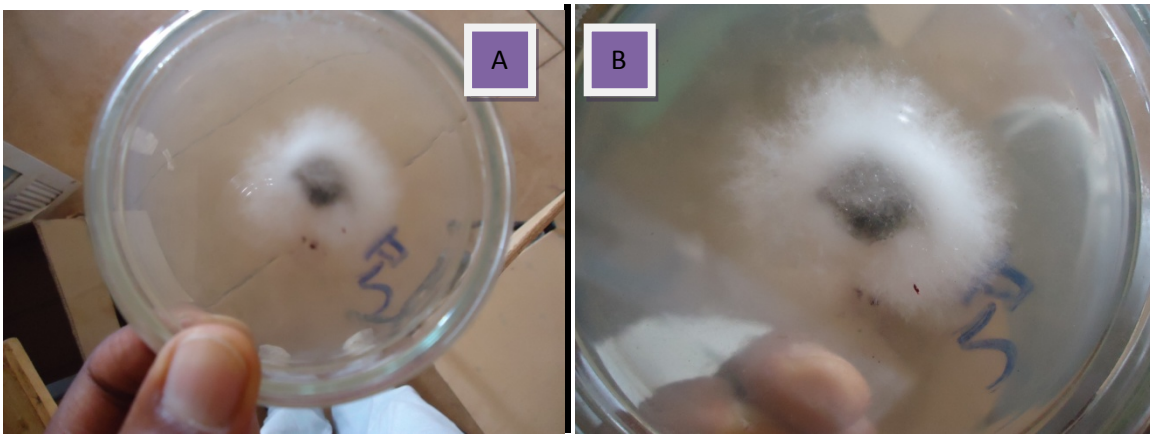


Figure 2. Petri dishes that are treated with a powdered: A/ *Senna occidentalis* extracts, and B/ *Melia azendrachta* extracts

4. Discussion

In this research the antifungal effect of the aqueous and ethanol (70%) extracts of five noxious plants were evaluated against *Colletotrichum kahawae* under in *in vitro* conditions. The findings showed that the effect of plant extracts on C. *kahawae* radial growth and disease development vary depending on the type of plant species

used, method of extraction, time of application, the type of formulation and part of the plant extracts applied. *Senna occidentalis* and *C. procera* were found to be the most effective plant extracts in inhibiting the radial growth of the pathogen in vitro condition. However, S. *occidentalis* is the most effective plant extract in reducing the radial growth of C. *kahawae invitro* in both aqueous and ethanol extracts. The result of this study corresponds with the report by [9] that sprays made from aqueous *Cassia spp* extracts have an antibiotic and antifungal activities/properties.

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [10] has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages[11]. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [12]. Arora and Kaur [12] have studied *S. occidentalis* L., exhaustively. However, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide [12, 14 and 15]. It is an ayurvedic plant with huge medicinal importance. *Senna occidentalis* leaf extracts demonstrated antimicrobial activity against both the test bacteria and fungi with water extract [16].

Previous investigations showed that *S. occidentalis* leaf extracts have antibacterial [17]. However, not many reports are available on the exploitation of antifungal of antibacterial property of plant products for developing commercial formulations for applications in crop protection.

Similar reports made by Tapwal, *et al.* [18] on the antifungal properties of some medicinal plants indicating that plant extracts are promisingly vital for the management of *C. kahawae.* In general a voluminous number of literatures reported the effectiveness of *S. occidentalis* against most pathogenic microorganisms. A crude extracts of *S. occidentalis*, however showed the highest inhibitory effect (<10%) at 10% concentrations against *Calotropis procera* and *Argemone mexicana*.

The effect of the antifungal compounds may be on spore germination leading to its inhibition of may be due to the effect of these compounds on the cell wall altering the permeability of [16]. The antifungal compounds may also suppress the early stages of mycelia growth leading to inhibition of the fungus. Next to Cassia, the dry, leaf extract of Parthenium had shown 58.73% growth inhibition against *C. kahawae*, followed by *Melia azadirachta*.

Similar findings on antifungal nature of parthenium were documented by [19] and [20]. Hence, by utilizing this weed in the plant disease management, one could protect the land from its invasion as well as get some economic gains by disease control. A number of antifungal compounds of diverse skeletal patterns have been found in the plants. These compounds belong mainly to six broad chemical groups, such as phenolics and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids and steroidal alkaloids, and other miscellaneous compounds [21]. However, only a few commercial products from the plant are being used in practical plant protection.

The fungitoxic effects of the phyto-extracts indicate the potential of selected plant species as a source of natural fungicidal material. Antifungal activity was confirmed by all of the selected plant species and the results revealed that different plant extracts varied in their efficacy for inhibiting the mycelial growth of tested pathogens. Although the selected concentration of tested plant species was unable to completely inhibit the pathogens but they could be used in combination with the fungicides as IPM strategy to minimize the use of fungicides. The finding of the present investigation could be an important step towards the possibilities of using noxious plant products as pesticides in the plant disease control.

5. Conclusion

This study evidenced that aqueous and ethanol extracts of *S. occidentalis* have potential to be applied as a control measure against coffee berry disease (*C. kahawae)*. The application of aqueous extract of *S. occidentalis* seems promising as it is simple, efficient and inexpensive alternative means of *C. kahawae* management for the farmers, which may replace the synthetic fungicides, particularly to those who cannot afford synthetic chemicals. Moreover, the risk associated with synthetic chemicals as well as consumers' resistance, towards its application in agriculture make the product more attractive natural product for organic agriculture. The findings also provide new scientific information on antifungal activity of S. *occidentalis* against *C. kahawae*.

Acknowledgements

The Authors are thankful to Wolaita Sodo University, Department of Biology for providing the entire materials needed for this research. Mr. Dejene and Mr. Ermias of Wolaita Sodo University deserve appreciation for their assistance.

References

[1] B. Tefestewold. "Studies on *Colletotrichum* population of *Coffea Arabica* L. in Ethiopia and evaluation of the reactions of coffee germplasms". Doctoral Dissertation. HohenLand wirtschaftlichen Fakultat der Rheinschen Friedrich-Wilelms-University, 1995.

[2] D. Paulos and T. Demel. "Coffee production systems in Ethiopia". in *Proceedings of the workshop on control of coffee berry disease (CBD) in Ethiopia*, 2000, pp 99-107. August 1999, Addis Ababa, Ethiopia.

[3] Ministry of Foreign Affairs-FDRE. Country Report. Addis Ababa, Ethiopia, 2012

[4] Q. Gray, T. Abu and T. Teddy. "Coffee Annual Report". Global Agricultural Information Network (GAIN Report). *USDA foreign Agricultural Report*, 2013, pp1-9

[5] A. Mesfin. "General introduction: An overview on the status of coffee, tea and spice in Ethiopia" in *Proceedings of the First Workshop on Production Constraints Assessment of Coffee, Tea and Spices*, 1991. pp. 15-17, September, Jimma, Ethiopia.

[6] D. Eshetu, G. Teame and A. Girma. "Significance of minor diseases of *Coffea arabica* L. in Ethiopia: A review" Pp 58-65. In: *Proceedings of the workshop on coffee berry disease (CBD) in Ethiopia*, 2000, 13-15 August, 1999, Addis Ababa, Ethiopia.

[7] A. Amsalu, L. Fikre and M. Diriba. "The antifungal activity of some medicinal plants against coffee berry disease caused by *Colletotrichum kahawae"*. *International Journal of Agricultural Research*, 2011, **6** (3): 268-279

[8] A.C. Amadioha. "Fungitoxic effects of extracts of *Azadirachta indica L.* against *Cochliobolus miyabeanus L.* causing brown spot disease of rice". *Arch. Phytopathol. Plant Prot*., 2002, **35**: 37-42.

[9] S. Deepika and S. Padma. "Antifungal Potential of Two Common Weeds against Plant Pathogenic Fungi-*Alternaria Spp"*. *Asian J.exp.biol.sci* . 2011, **2**(3): 525-528

[10] I.C. Zampini, S. Cuello, M.R. Alberto, R.M. Ordonez, R.D. Almeida, E. Solorzano, and M.I. Isla. "Antimicrobial activity of selected plant species from the Argentine puna against sensitive and multi-resistant bacteria". *Journal of Ethnopharmacology*, 2009, **124**: 499-505.

[11] P.O. Kemo, H.P. Bais, and J..M. Vivanco. "*In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens". *Fitoterapia*, 2003, **74**: 312-316.

[12] T.S. Roopashre and D. Raman, R.R.H. Shobha and C. Narendra. "Antibacterial activity of antipsorriatic herbs: Cassia tora, *Momordica charantia* and *Calendula officialis"*. *International Journal of Research in National Products*, 2008, **3:** 20-28.

[13] D.S. Arora, and G.J. Kaur. "Antibacterial activity of some Indian medicinal plants". *Journal of Natural Medicine*, 2007, **61**:313-317.

[14] O.F. Kunle and H.O. Egharevba. "Preliminary studies on *vernonia ambigua*: Prhytochemistry and Antimicrobial screening of the whole plant". *Ethnobotanical Leaflets*, 2009. **13**: 1216-21.

[15] A. O., Ettu, C. T. Senjobi, and O. A. F. Ilusanya. "Antimicrobial Activity of Leaf Extracts of *Senna occidentalis* (L)". *Journal of Agri. and Bio. Sciences* Vol. **2**(1), 2012, pp. 026-030, http://www.globalresearchjournals. com/ journal/id=JABS/homeid=JABS

[16] Jaya, J. "Studies on paradoxial nature of Indian herb: *Azadirachta indica"*. International Journal of Chemical Sciences, 2008, 6 (3): 1571-1575.

[17] S. A. Khan, and J. Aslam. "Study on the Effect of Neem (*Azadirachta indica*) Leaves Smoke in Controlling Airborne Bacteria in Residential Premises". *Current Research in Bacteriology*, 2008, 1 (2): 64-66.

[18] A. N. Tapwal, G.Shipra, G. Nandini, and K. Rajesh. "*In vitro* Antifungal Potency of Plant Extracts Against Five Phytopathogens". *International Journal of pl. pathol.* , 2011, **54** (6): 1093-1098

[19] R. Bajwa, S. Shafique, T. Anjum, and S. Shafique. "Antifungal activity of allelopathic plant extracts IV: Growth response of *Drechslera hawaiiensis, Alternaria alternata* and *Fusarium moniliforme* to aqueous extract of *Parthenium hysterophorus"*. *Int. J. Agri. Biol.* , 2004, **6**: 511-516.

[20] N. Sharma and P. C. Trivedi, "Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum"*. *Asian J. Exp. Sci.*, 2002. 16: 2128.

[21] S. R. Mitra, A. Choudhuri, and N. Adityachaudhury. "Production of antifungal compounds by higher plants-a review of recent researches". *Plant Physiol. Biochem*. , 1984, **11**: 53-77.